



Artificial insemination in pig, its status and future perspective in India: A review

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ABSTRACT

The pig production must be sustainable, efficient and competitive to minimize its impact on environment. There is argument to keep fewer breedable animals to produce more numbers of piglets. In India, pig rearing is still done on traditional manner with low productivity. Artificial insemination (AI) is the best reproductive technology available in the current scenario for enhancing the efficiency of pig production in a sustainable way. This in pig involves, collection of semen, processing in laboratory, packaging in 80 to 90 ml semen pouch containing two to three billion spermatozoa and inseminating the sow at correct time. AI in pig is used widely in the world with the use of freshly diluted semen. The use of AI has allowed significant improvement in swine population over the world. Besides genetic improvement, it allows for better maintenance of farm record, saving farm labour and prevents disease transmission. Artificial insemination in northeast India has been introduced only recently but the response from the different stakeholders is quite favourable. In order to replicate the successes achieved in different countries, a proper understanding of the technique, its adaptability to local conditions and the regulations need to be put in place. The establishment of support services is vital to achieve success. The technology has the potential for enhancing the profitability of pig farming in sustainable way. To reap the full potential of this technology, concerted efforts are needed from all stakeholders.

Keywords: Artificial insemination, Boar, Liquid semen, Pig

The demand for pork is continuously increasing owing to increase in human population. The pig production must be sustainable, efficient and competitive to minimize its impact on environment. There is argument to keep fewer breedable animals to produce more numbers of piglets (FAO 2003). In India, pig rearing is still done on traditional manner with low productivity (Singh *et al.* 2019a). Artificial insemination (AI) is the best reproductive technology available in the current scenario for enhancing the efficiency of pig production in a sustainable way (Singh *et al.* 2019b). The Artificial insemination is the reproductive biotechnology in which semen is collected from the male, processed in the laboratory and deposited artificially into female reproductive tract. Artificial insemination is a widely adopted technology in breeding programmes for livestock improvement since the late 1940s (Singh *et al.* 2014). This has been made possible due to the fact that spermatozoa can be collected, processed, stored and deposited in the reproductive tract of female animals. The technology has been standardized for most of the domestic livestock species with species-specific modifications. This is being widely used in cattle, buffalo, sheep, goat and pig in all over the world. AI in pig is widely used on commercial scale in the countries with intensive pig rearing (Maes *et al.* 2011). It is

being used in more than 90% pig in Western Europe (Gerrits *et al.* 2005). However, in India, AI is used most mostly in cattle and buffalo only and its use in pig has been restricted to the academic and research purposes despite its perceived benefits. In India, hardly 1 to 2% of breedable pigs are covered by artificial insemination. Introduction of new germplasm over a short period of time, extensive use of superior boars, disease control and extensive record keeping are some of the benefits of artificial insemination over the natural breeding (Singh *et al.* 2018). Farrowing rate of 86% and litter size of 14 piglets had been reported by use of AI in pig (Yeste *et al.* 2014; Singh *et al.* 2018). It is an extremely easy and low cost technique and holds great promise for the Indian pig farmer. AI in pig involve use of freshly diluted liquid semen, transported on same day of collection or stored at 17°C for three to ten days before use depending upon the extender (Singh *et al.* 2018a). Currently, due to long uterine horn of pig and ovulation over a long period of time; two to three billion spermatozoa are used in each AI dose. Although, there is much progress made in cryopreservation of pig semen, however, low farrowing rate and small litter size with use of cryopreserved semen limit its use to research purpose only. The current paper discusses in detail the different aspects of AI technology in pig, its status of adoption and future perspective in India.

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Boar Training

Boar training is most important part in the whole process

and many a time it may turn out in a frustration for the farm manager. Success of AI programmes mainly depends on genetic makeup of boars and quality of semen. Male piglets of superior pedigree are selected at weaning and kept in groups away from female animals. Further selection is done at the age of 6 to 7 months on the basis of growth rate and body conformation. At this age boars are kept in isolation and at least 50 to 100 feet away from female shed. Boar training is done in the morning hours before feeding. The young boars are introduced to the dummy and allowed to sniff the dummy which may be smeared with the saliva, urine or vaginal discharges of sows in heat. Otherwise the saliva and semen of older boars may also be used. Dummy should be solid in construction (made up of wood or metal) without sharp edges, and located in a quiet designated semen collection room with a non-slippery floor (Maes *et al.* 2011) or having provision of rubber mat. Prior to training, dummy mounting and semen collection of older boars may be done in view of the younger boars as this influences a positive response in faster mounting by the trainee boars. Generally the boar should start mounting the dummy within 15 days and start donating ejaculates within 20 to 25 day (Knox 2016). However, sometimes it may take four to five months depending upon the libido of the boar and experience of the trainer (Singh *et al.* 2018a). Boar displaying low libido should be rejected from breeding programme. Semen should be collected at minimum intervals of at least 3 days for optimum semen fertility (Singh *et al.* 2018a).

Semen collection

In boar, semen is collected by the gloved hand method in which the cork screw tip of the boar penis is grabbed and held by the hand of the collector using a polyvinyl glove (do not use latex gloves) (Ko *et al.* 1989). In glove hand technique, applying pressure on the cork screw part of penis by hand is major reflex for boar to ejaculate. The semen is collected into a collection flask or thermos flask (maintained at 37°C) through a Buchner funnel fitted with milk filter to filter out the gel plug of the boar ejaculate. The pre-sperm fraction (about 10–20 ml) consisting of preputial fluid and urine accumulated in the prepuce is discarded and only the sperm rich fraction (100 to 400 mL) is collected (Maes *et al.* 2011). The ejaculation period generally last for five to ten minutes. The post-sperm rich part is watery and clear fluid which should be discarded. Also, filter along with gel should be discarded. The collected ejaculate is brought to laboratory within 15 min for processing and dilution. All the materials and equipment which comes into direct contact with semen must be sterile to avoid the bacterial contamination. During the whole process of semen collection and processing, the temperature of the semen collection flask, extender and other materials should be kept at 37°C.

Semen quality parameters

The success of AI programmes is mainly due to the fact that boar ejaculates could be evaluated in laboratory for

its quality. The colour of the semen should be creamy white and free of any debris and blood. The opacity of ejaculate roughly indicates the concentration of spermatozoa. Volume of ejaculates varies from 100 to 400 mL depending upon the breed, climate and season (Kommisrud *et al.* 2002). In Indian condition, the volume of Gunghroo and Hampshire crossbreed boar semen generally varied from 100 to 300 mL and it is more during winter season (Singh *et al.* 2019c, Singh *et al.* 2019d). Feeding of flaxseed oil significantly enhanced the boar semen volume from 250 mL in controlled animal to 350 mL in treated animals (Singh *et al.* 2019c). Sperm concentration in boar semen ranges from 100 to 300 million sperm cells per ml of ejaculate and 30–60 billion sperm cells per ejaculate (Singh *et al.* 2019c). Sperm concentration is determined by haemocytometer, thoma cell counting chamber, photometry, flow cytometry or by computer assisted semen analysis (CASA). Motility of spermatozoa is a primary requirement to fertilize eggs successfully. Spermatozoa are brought to the fertilization site mainly by uterine contractions, however, sperm motility is required for penetration of the zona pellucida (Langendijk *et al.* 2006). Initial sperm motility in boar is determined by subjective assessment at 400X under cover slip in light or phase contrast microscope maintained at 37°C or objective assessment by CASA. Ejaculates having motility of more than 70% are selected for further processing for use in AI programmes (Flowers, 2009). Variation in sperm motility has been linked to differences in pig fertility (Foxcroft *et al.* 2008), particularly when sperm motility falls below 60% (Flowers 1997). During first few ejaculates, there is high percentage of proximal and distal cytoplasmic droplets in pig spermatozoa which goes on decreasing with the time. Higher percentage of sperm with cytoplasmic droplet indicate immature spermatozoa which are present during initial stage of collection or this may also be due to higher frequency of collection. Generally, initial sperm motility varies from 70 to 90% in fresh boar semen (Singh *et al.* 2019c). However, this goes on decreasing with the time on storage in liquid condition depending upon the extender used. Different workers have suggested cut off value for different parameters for boar semen to be included in AI programme, viz. motility more than 70%, cytoplasmic droplets less than 20%, primary abnormalities less than 10% and secondary abnormalities less than 20% (Britt *et al.* 1999). The microscopic appearance of spermatozoa can give information on morphological abnormalities, cell membrane integrity and the acrosome. These are three important parameters that contribute to the fertilizing capacity of the sperm cells (Maes *et al.* 2011). Eosin-nigrosin is the most common dye used for viability as well as morphological assessment of spermatozoa. Primary abnormalities, particularly of head and mitochondrial sheath are important and these cannot be compensated by enhancing the semen dose. Boar's ejaculate having primary abnormalities should be rejected from the AI programmes. Recently, fluorescent dyes like FITC-PSA/PNA etc. are

being used for spermatozoa viability as well as acrosomal intactness. Acrosome is an important part of sperm playing a predominant function during fertilization process. Acrosomal intactness is assessed by giemsa stain (Watson 1975). Spermatozoal membrane integrity is being assessed by hypo-osmotic sperm swelling test (Jeyendran *et al.* 1984). These are the few routinely used sperm quality parameters for monitoring semen quality. There are other *in vitro* as well as *in vivo* tests available for comprehensive evaluation of semen, however, these are not in routine use. The best test for prediction of fertility is pregnancy. Although, there are a number of tests available for assessing semen quality, it should be kept in mind that no single test accurately predicts the fertility outcome. Therefore, a number of tests is used generally for identifying the poor ejaculates.

Semen processing and preservation

Boar spermatozoa differ from other species in membrane composition particularly having low cholesterol to phospholipid ratio and an asymmetric al distribution of cholesterol within the membrane which make it very susceptible to cold temperatures resulting in increased membrane permeability and loss of controlled membrane processes (De Leeuw *et al.* 1990). Due to this, cryopreserved boar semen yields very low conception rate and litter size. The supplementation of butylated hydroxytoluene alone or in combination with cholesterol loaded cyclodextrin showed a significant increase in post-thaw motility as compared to control (Baishya *et al.* 2018), however, there is still a long way to use cryo-preserved semen for commercial purpose in pig breeding. As a result, liquid boar semen is used globally for AI on the day of collection or in the following days.

Semen is collected in pre-heated semen collection flask or in thermous flask. The extender is dissolved in double distilled water at 35–37°C and the semen is mixed with it in single step. Packaging is done in semen pouches made of neutral non-spermicidal material. The minimum sperm concentration per AI should be at least 2–3 billion spermatozoa in 80–100 ml of insemination volume (Knox *et al.* 2008). From a single ejaculate around 10 to 15 semen doses are made. The processed semen is preserved at 16–18°C in BOD incubators. Metabolism of spermatozoa reduced at this temperature, a condition necessary for extending the semen storage time. Different semen extenders have different storage period. Now days, commercial semen extenders are available in market for different duration, i.e. up to three to 12 days. Short term extenders that is up to three days are most commonly preferred because of economic aspect, closeness of farm to boar station and quality of semen. Boar semen extender contains energy source, ions for maintaining osmotic pressure, buffering agent for stabilizing pH, antibiotic, chelating agent and membrane stabilizer. Different extenders of boar semen also affect the seminal attributes on storage at 16–18°C (Khan *et al.* 2012).

Oestrus detection and insemination

After the successful collection and processing of semen, the sow/gilt must be inseminated. The correct timing of insemination is vital to reaping the full benefit of doing AI (Singh *et al.* 2018a). Estrus duration and its control, timing and number of inseminations, the AI procedure, semen storage on farm and the use of new AI technologies, require a thorough knowledge of pig reproductive physiology (Maes *et al.* 2011). Regular detection of estrus at the farm with the help of boar aids in correct and accurate heat detection. Presence of boar leads to enhanced follicular development and thereby expression of oestrus behaviour (Langendijk *et al.* 2006). Boar stimuli increase the frequency of uterine contractions due to increase of oxytocin concentrations in peripheral blood plasma (Langendijk *et al.* 2006). The average length of oestrus varies mainly due to parity, season, weaning-to-oestrus interval and farm management. The goal of heat detection is to determine when the sow or gilt reaches standing heat. Standing heat is the period when a female stands still and rigid when weight is applied on her loin. Lordosis or standing heat is identified by signs such as swelling and reddening of the vulva, vulvar discharge, vocalization, in-appetence, boar-seeking behaviour, ear popping and standing for back pressure (Singh *et al.* 2018a). The duration of oestrus in sow lasts from 48 to 72 h. After weaning the sow may return to heat by 4–6 days (Yeste *et al.* 2014). In gilts the heat lasts for 40–48 h. Two inseminations during standing oestrus at an interval of 12 h is the preferred strategy, however, in few sows a third insemination is also done if oestrus prolongs beyond 72 h (Knox 2016; Singh *et al.* 2018a). Gilts should be bred 12–24 h after heat is detected, and again 12 h later. Sows should be bred 24–36 h after detection of heat, and again 12 h after the first insemination. The ovulation in pigs occurs in the later 2/3rd part of the heat. Generally the farrowing rates are 70 to 85% when the semen is used in the first 2 days after collection (Singh 2018). Optimal AI schedules are those that result in a high farrowing rate and litter size, while minimizing costs of semen and labor by avoiding unnecessary inseminations. Significantly higher litter size at birth and at weaning was recorded by post-cervical artificial insemination compared to traditional AI (Singh *et al.* 2019d). The site of semen deposition determines survivability, litter size and conception rate. Semen is deposited at the outer end of the cervix during natural mating and conventional AI.

The procedure of AI should be performed in neat and clean environment and taking into consideration all the sanitary measures including cleaning of the vulva to remove urine and feces and use of a new AI catheter. For AI, the tip of the catheter is lubricated with non-spermicidal gel and inserted into the vagina for passage into the cervix while rotating anti-clockwise. After locking of the catheter in the cervix, the semen pouch is attached to the catheter and the semen is allowed to flow into the cervix using gravity and gentle pressure over a 3- to 4-minute period. The catheter is inserted at an angle of 30° to the backbone.

Simultaneously rubbing the flank and underlying region stimulate the female to suck semen into the uterus. After complete deposition of the semen, catheter is gently withdrawn by simultaneously twisting it in a clockwise direction. Insemination to ovulation interval has a great effect on fertility outcome mainly due to effect on sperm reservoir, egg fertilization, and embryo survival (Cornou 2006). Optimal fertility is reported with insemination occurring 12 hours before ovulation (Cornou 2006). Recently, enormous progress has been made to extend the use of superior sires including reducing the number of sperm in the AI dose (Vazquez *et al.* 2008), use of a post-cervical artificial insemination (PCAI) or intra-uterine insemination (Singh *et al.* 2019d), and reducing the need for multiple inseminations by control of ovulation and single, fixed time AI or AI with oestrus synchronization (Knox 2015). The greatest numbers of pigs could be produced when using 2.5 billion sperm with insemination at 22–26 h. Fertility with single, fixed time insemination is influenced by stage of ovarian follicle development, expression of oestrus, and sow parity (Knox *et al.* 2017). Advances in AI technology are to be in sync with new sperm technologies, such as the use of lower number of sperm per AI dose, because the traditional AI has a very low efficiency (Singh *et al.* 2019d). Deposition of sperm near the site of fertilization in the oviduct is being actively pursued to enhance the efficiency. Using deep intrauterine insemination with a specially designed catheter, a 20-fold reduction in the number of freshly and diluted inseminated spermatozoa can be achieved without compromising the fertility (Vazquez *et al.* 2005).

Advantages of AI in Pig with reference to India

Indian pig farming constitutes the livelihood of rural poor people belonging to the lowest socio-economic status. Tribal farmers rear pigs- under scavenging system in major parts of India and under backyard production in North-eastern India (Singh *et al.* 2019a). The bulk of the pig population in India is of indigenous type with poor growth rate and productivity (Kumaresan *et al.* 2006). Pig production has a high potential to contribute to higher economic gain as pigs have higher fecundity, higher feed conversion ratio, early maturity, shorter generation interval and relatively smaller space requirement (Singh *et al.* 2018). However, pork production in India is limited, representing only 7% of the country's animal protein sources. Consumption of pork is concentrated mainly in the north eastern states of the country and there a huge demand and supply gap exists (Kadirvel *et al.* 2012, Singh *et al.* 2018). Farmers keep pig mainly for meat purpose. There is lack of interest among farmers for pig rearing for breeding purpose mainly due to non-availability of breeding boar, high cost of breeding, non-availability of field methods for pregnancy diagnosis and lack of awareness among the farmers (Kadirvel *et al.* 2012, Kumar *et al.* 2017). In NEH region, tribal farmers generally keep one boar for 4–5 villages for breeding purpose and they have to transport female pig at the time of oestrus which

further adds cost to the farmers (Singh *et al.* 2019b).

For all the above mentioned felt needs, artificial insemination is the easiest way to improve the local germplasm in the shortest way with very low input costs. Compared with natural mating, artificial insemination is the better way to introduce superior genes into sow herds, with a minimal risk of disease. The genetic selection programme in pig based on AI helped in improving economic traits such as growth rate, higher feed conversion efficiency, carcass trait, mothering ability and litter size. AI in pig is widely practiced throughout the world and is a very useful tool to introduce superior genes into sow herds, with minimal risk for disease transmission (Maes *et al.* 2011, Knox 2016). Initially AI was more focused on to improve breeding management and preventing the spread of venereal diseases rather than a mean to accelerate genetic progress. Artificial insemination coupled with oestrus synchronization is very useful in planned and controlled breeding. Artificial insemination also helps in avoiding inbreeding due to repeated use of same boar in natural mating. It will also eliminate the need of keeping boar at every farm or household for breeding purpose which will lower the cost of production (Singh *et al.* 2019b). Also, AI helps in better maintenance of record at the farm. Semen from a single ejaculate can be used for breeding 10 to 20 females. With the availability of long term extenders, AI in pig has been adopted on a wider scale (Knox 2016). Long term extender could be of immense help in country like India having hilly terrain, sparsely located pig population in NEH region, and less-developed transportation systems. In addition to the above mentioned benefit, AI helps in control of venereal diseases. It will allow maintenance of close nucleus herd and therefore prevent entry of diseases. AI allows better maintenance of record and save the labour involved in natural mating. Separate semen production centre helps in better boar management and leaving the oestrus management for breeder farm. This also, allows better boar health management, housing, and feeding. Early semen collection from a boar allows comprehensive semen examination in laboratory. Therefore, early culling of sub-fertile boar can be done. Therefore, large-scale application of Artificial Insemination (AI) technology in pig under field conditions and horizontal spread of superior germplasm needs to be taken up expeditiously.

Besides the numerous advantages of AI, there are a few issues associated with it which need adequate attention. The semen can act as a carrier of diseases. However, most of the micro-organisms that have been detected in boar semen are considered non-pathogenic, but some are known pathogens (e.g. porcine reproductive and respiratory syndrome virus) that can cause major economic losses (Maes *et al.* 2008). Microbial contamination of semen can be due to infected boar, or may be due to inappropriate handling of semen. Presence of pathogens leads to accumulation of reactive oxygen species in semen which further deteriorates the quality of spermatozoa. It can result in embryonic or foetal death, endometritis, pyometra, repeat

breeding and systemic infection and/or disease in the recipient female (Maes *et al.* 2008). To prevent the pathogen contamination of semen, it is necessary to maintain specific pathogen free boars and maintain very strict biosecurity at AI station. Additionally, cocktail of antibiotics are added in extender for reducing contamination during semen collection, processing, and storage.

Artificial insemination technology in pig in India

The AI of swine was initiated by Ivanow in Russia in the early 1900s. More than 90% of sows are bred by AI in Western Europe (Gerrits *et al.* 2005). In the USA too, more than 70% pigs were bred through AI in the year 2000. Despite the world scenario, AI in pig in India has not yet received adequate attention due to lack of awareness among the farmers, policy makers and there is inadequate infrastructure facilities at field level (Singh *et al.* 2019d). AI in pigs in India has mostly been just at an academic interest level until recently. National Research Centre on Pigs (NRC), Rani, Guwahati successfully introduced AI technology at field level in Assam. Artificial insemination in Nagaland is being undertaken successfully under ICAR-Mega Seed Project on Pig (Singh 2018). Recently, there is a renewed impetus under AICRP on pig breeding for adoption of AI at farm level. However, except for north east India, there are no reports of using AI in pig at field level. Non availability of basic infrastructure support like electricity, distilled water, BOD incubator at the field levels are the specific reasons for non adoption of AI in pig. Also, lack of technical knowledge at the field level poses a major hindrance for spread of this technology. Singh (2018) recorded farrowing rate of 89.48% in farmer's field in Nagaland with average litter size of 10.06 with the use of AI. AI in pig has immense potential in empowering the tribal farmers and developing them into potential entrepreneur (Singh *et al.* 2017). ICAR Nagaland has carried out a total of around 2500 inseminations from 2013 till 2019 and produced around 20000 piglets of improved germplasm. The average litter size has increased from 7.15 to 10.21 as compared to natural mating. The farrowing rate has also stayed at an average of around 80% through this period. The cost of AI is also 1/10th of the cost of natural service in Nagaland. Due to limit on storage time of liquid boar semen, adoption rate of AI is higher in districts near to ICAR farm in Nagaland (Singh *et al.* 2019b). In Mizoram, pigs farming cooperative societies are carrying out AI extensively and is becoming an enormous success. It is also the first state in the country to start AI programme. ICAR Umiam is also carrying out AI in pig in Meghalaya. However, In India, hardly 1–2% of breedable pigs are bred through artificial insemination. Kadirvel *et al.* (2012) observed the following benefits of AI for tribal farmers', viz. timely availability of superior germplasm for breeding, economical in comparison to natural breeding and prevention of inbreeding. In addition to genetic improvement of nondescript local pigs, AI can help in overcoming breeding constraints faced by tribal farmers

who practices low input backyard pig farming. There is urgent need to take up this technology to the farmers.. Specifically, there is need to develop the basic infrastructure at ground level for adoption of AI in India. Establishment of satellite AI centre in remote areas in collaboration with main boar station is the need of hour.

CONCLUSION

AI in pig is used widely in the world with the use of freshly diluted semen. The use of AI has allowed significant improvement in swine population over the world. Besides genetic improvement, it allows for better maintenance of farm record, saving farm labour and prevents disease transmission. Artificial insemination in northeast India was introduced only recently but the response from the farmers is quite favourable. It has already taken off in parts of Nagaland, Assam, Meghalaya and in Mizoram with enormous success. In Nagaland, ICAR has introduced this technology and is getting popular among farmers. In order to replicate the successes achieved in different countries, a proper understanding of the technique, its adaptability to local conditions and the regulations that need to be put in place. The establishment of support services is vital to achieve success. The technology has the potential for enhancing the profitability of pig farming in sustainable way. To reap the full potential of this technology, concerted efforts are needed from all stakeholders.

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